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DETERMINATION OF A UNIQUE EPITOPE BINDING SITE FOR A COMPLEMENT-LYSIS-ENHANCING MONOCLONAL ANTIBODY, 3D12, ON THE GALACTOSE ADHERENCE LECTIN OF ENTAMOEBA HISTOLYTICA, USING BIAcore

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13. ABSTRACT *(Maximum 200 words)*

Mechanisms of pathogenicity used by Entamoeba histolytica to invade the bloodstream and cause liver abscess, include complement mediated lysis. Although pathogenic strains of E. histolytica become involved in immune functions by (1) activation of the alternate complement pathway, (2) binding of the C9 component of complement, and (3) depletion of active complement mediated hemolysis, the organism is not eliminated from the body by complement induced lysis. Monoclonal antibody (mAb) 3D12 was produced to target molecules responsible for lysis resistance. Binding of mAb 3D12 to the galactose-specific surface lectin, circumvented resistance to C5b-9 complement lysis. The epitope position on the adherence lectin for mAb 3D12 was mapped using BIAcore. Epitope specificity of mAb 3D12 is unique and separate from six previously characterized monoclonal antibodies and plays a role in enhancing complement mediated lysis of E. histolytica.

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PREFACE

The work described in this report was authorized under Contract No. DAAD05-91-P4347. This work was performed at the U.S. Army Chemical Research, Development and Engineering Center (CRDEC) from June 1991 to September 1991. Experimental results are on file in the Research Directorate, CRDEC, laboratory notebook 91-0036.

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1. INTRODUCTION

BIAcore™ (Pharmacia Biosensor, Piscataway, N.J.) uses surface plasmon resonance to detect changes in a sensor surface as bio-molecular reactions occur. Changes in light reflection are a direct result of changes in mass occurring at the sensor surface. The reaction surface can be made biospecific by immobilizing the ligand of choice onto a derivatized dextran layer. Controlled sequential injections of reactants makes it possible to measure each binding event using no molecular labels.[1]

A reaction surface is prepared in BIAcore by covalent coupling of a ligand to a derivatized matrix of dextran located on a sensor chip. This reaction surface, attached to a 50nm gold surface, is supported by glass. Monochromatic light, directed at the metal surface, is reflected by total internal reflectance. Concomitantly, evanescent wave energy from within the metal surface is generated and results in a portion of minimally reflected light. The change in the angle of minimally reflected light is a direct result of mass changes on the metal surface. This change is monitored electronically and recorded graphically as reactions occur. A response, expressed as resonance units (RU), of 1000 roughly corresponds to 1 ng per mm^2 attached monoclonal antibody. Other corresponding weights may vary with the protein used, depending on its complexity.[1]

E. histolytica causes parasitic infections worldwide and approximately 110,000 deaths per year. It is found in South and Central America, Africa, India, and the United States and surpassed only by malaria and schistosomiasis as parasitic causes of death.[2] A search of DOD technical report summaries revealed 82 citations for studies performed in at least 8 different countries.[3] In addition, 25 unclassified U.S. government DA, DF, and DN projects were documented.[4]

E. histolytica carries a 170 kDa adherence lectin responsible for causing disease. The lectin inhibits C5b-9 complement activity on pathogenic strains. A recently generated monoclonal antibody, 3D12, partially decreased inhibition by the adhesin and increased amebic susceptibility to lysis in the presence of normal human serum. When purified human complement, C5b-9, was added, lysis increased threefold. This lysis was not reproduced when heat inactivated (complement-free) serum was used. MAb 3D12 served to neutralize circumvention of lysis by

the amebic surface. It was shown using immunoprecipitation and Western blots that mAb 3D12 recognized the 170 kDa subunit of the adherence lectin.[5]

The objectives of this study were to more clearly define pathogenic mechanisms of E. histolytica by 1) structuring an affinity profile for the binding of 6 monoclonal antibodies to the adherence lectin, 2) substituting mAb 3D12 to estimate its point of attachment in reference to the other 6 monoclonal antibodies, and 3) target portions of the adherence lectin that are responsible for circumvention of complement mediated immune elimination in pathogenic strains.

2. MATERIALS AND METHODS

Purified adherence lectin, purified monoclonal antibodies and mAb 3D12 in ascites fluid were obtained from Dr. William Petri, Jr., University of Virginia, Charlottesville, Va. Buffers used in the procedures included immobilization buffer, (10mM sodium acetate, pH 5.0), running buffer, HBS, (10mM Hepes, 150mM NaCl, 3.4mM EDTA, and 0.05% surfactant P20, pH 7.4), and HBS with no salt. Standard immobilization techniques for attachment of amino groups to a carboxyl rich surface were used. The reagents, n-hydroxysuccinimide, n-ethyl-n'-(dimethyamino-propyl) carbodiimide, and 1.0 M ethanolamine hydrochloride, pH 8.5, were supplied by Pharmacia Biosensor. In addition, rabbit anti-mouse Fc antibody was supplied by Pharmacia Biosensor.

Purified lectin was used at a concentration of 50 ug/mL in immobilization buffer for attachment to the dextran surface. Purified monoclonal antibodies were used at concentrations of 20 ug/mL and added in succession for three separate runs to ensure reproducibility of stacking response. In addition, the order of injection of the antibodies was varied to establish a relative sequence of lowest to highest quantity attachers.

Due to the reluctance of purified mAb 3D12 to attach to lectin, preparations of mAb 3D12 in ascites fluid were tested for their propensity to bind to antigen. For this set of experiments, the attachment base was varied. Rabbit anti-mouse Fc (RAM-Fc) antibody was immobilized to the dextran surface and used to capture 3D12 from the ascites fluid. Following a wash step and blocking of all unreacted sites of RAM-Fc by an unrelated antibody, antigen was introduced. This prepared the surface for performing a stacking response using all seven monoclonal antibodies and RAM-Fc as a base.

3. RESULTS

A resonance response of 12571 RU was obtained from immobilization of antigen which served as a base for monoclonal antibody attachment. Figure 1 shows the relative stacking

response of purified preparations of the monoclonals. MAb 3D12 did not attach in its purified form. The graph represents an average of 3 values for each monoclonal from three separate runs. When the order of injection of the monoclonals was varied, the same relative stacking response was obtained. (Data not shown)

Figure 2 shows a change in response when mAb 3D12 was included in the sequence. Using RAM-Fc as a base, mAb 3F4 was used to capture antigen and the remaining 6 monoclonals were successively added. The graph shows lower relative binding of mAbs 7F4 and 8A3, and higher relative binding of mAb 3D12. In Figure 3, the relative order of binding, highest to lowest responder, shows that mAb 3D12 partially displaces both mAbs 8A3 and 7F4.

Figure 1. Stacking response of purified monoclonal antibodies on the adherence lectin of Entamoeba histolytica.

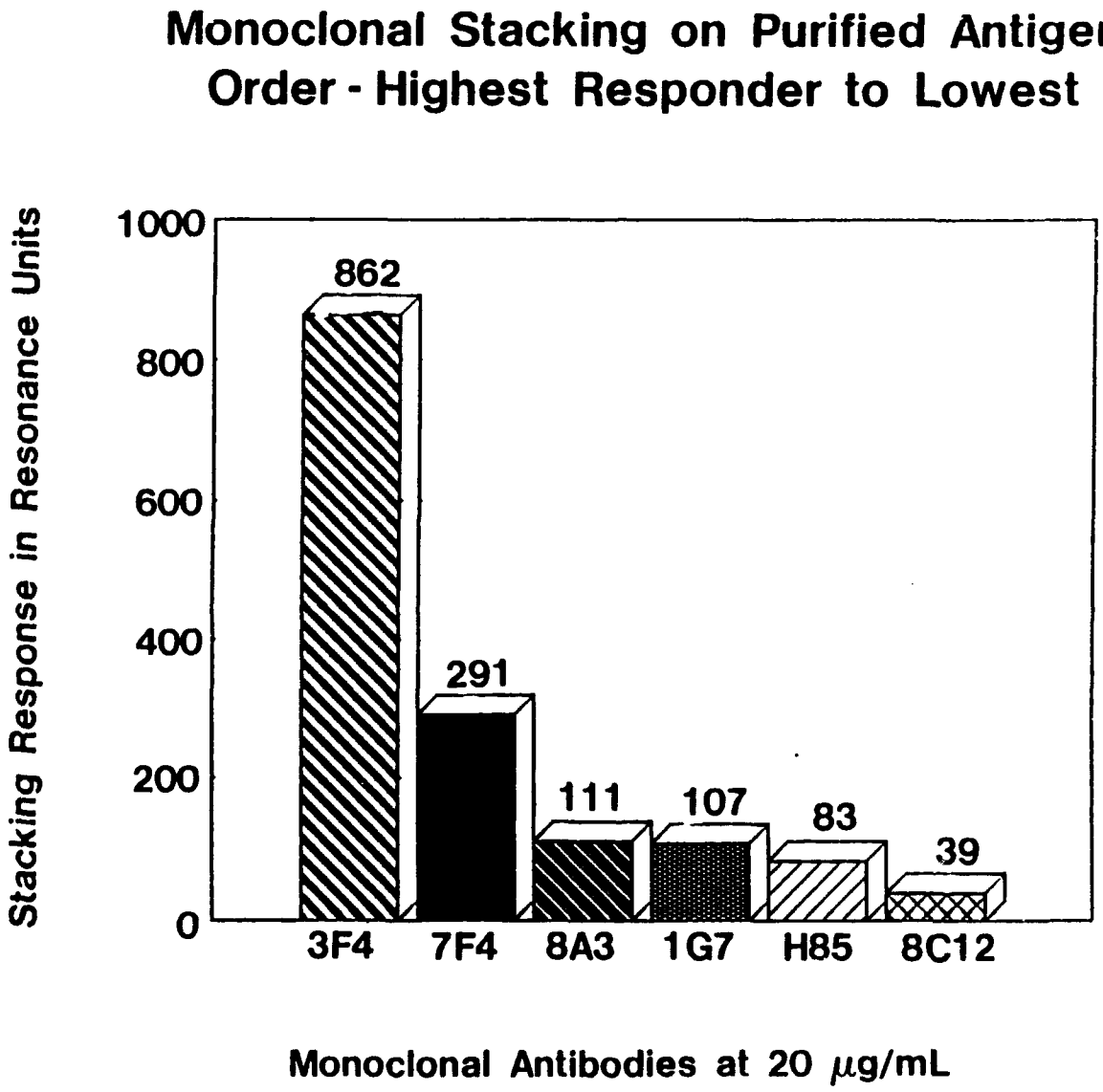


Figure 2. Change in relative stacking response with the addition of mAb 3D12.

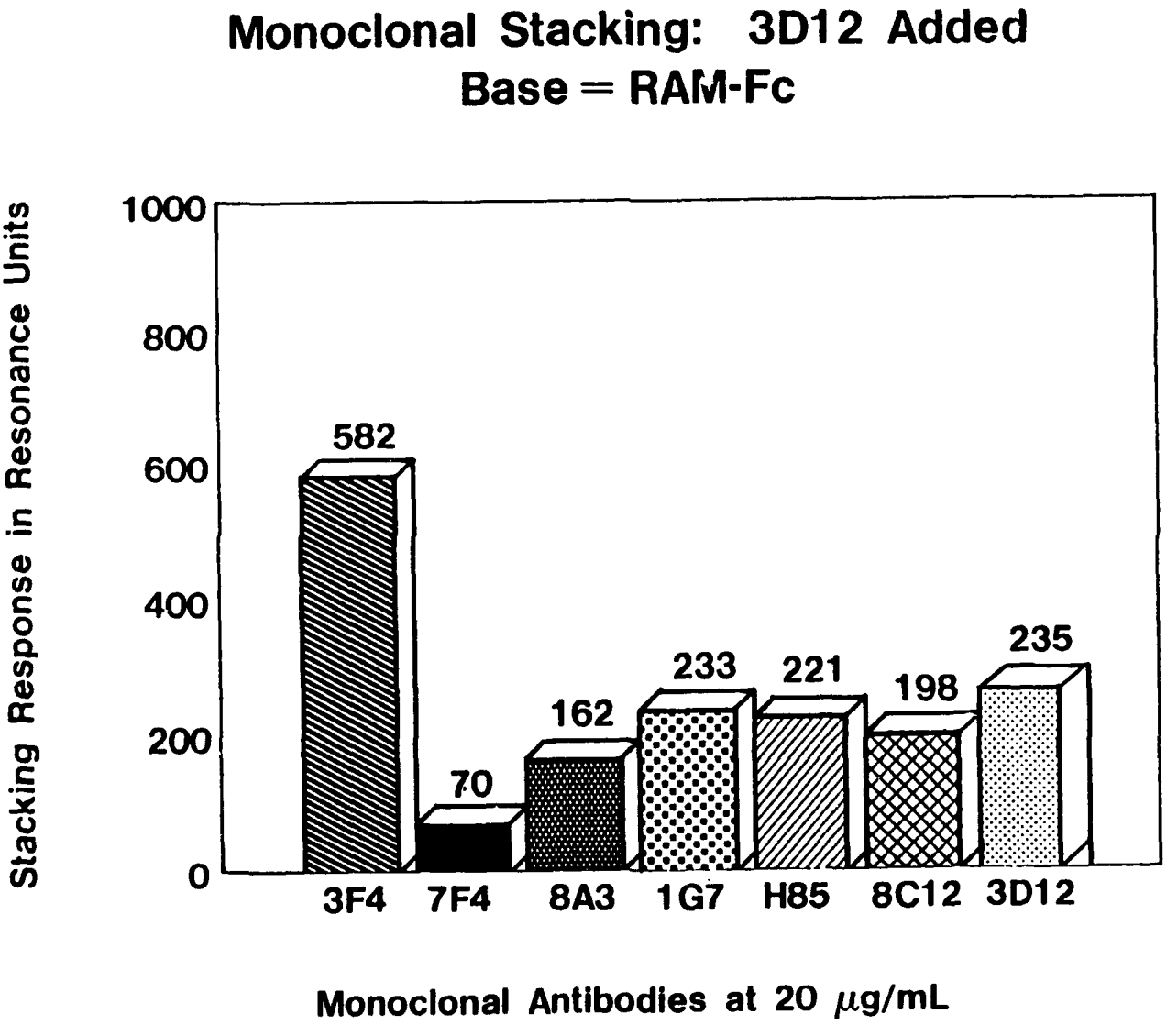
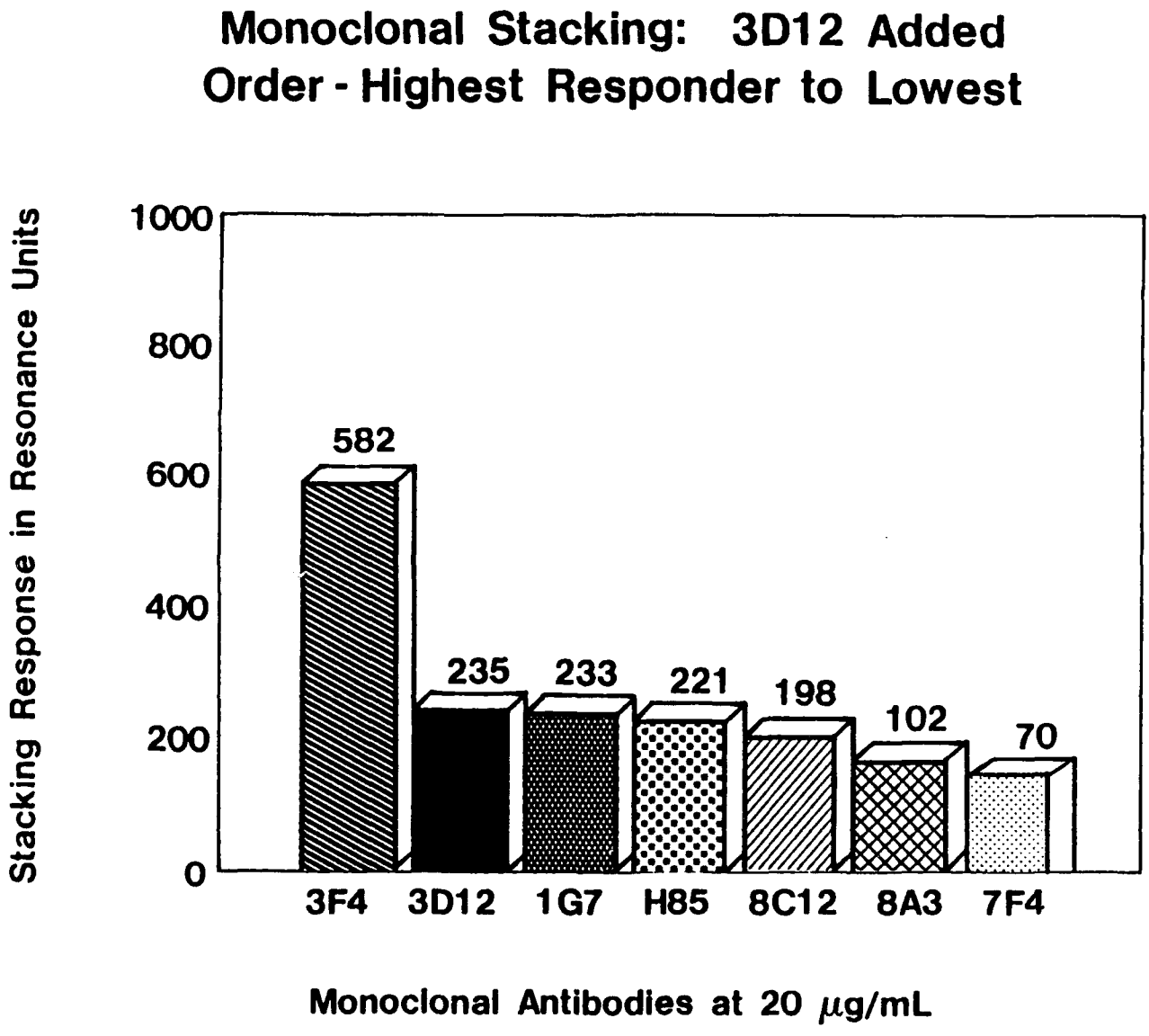


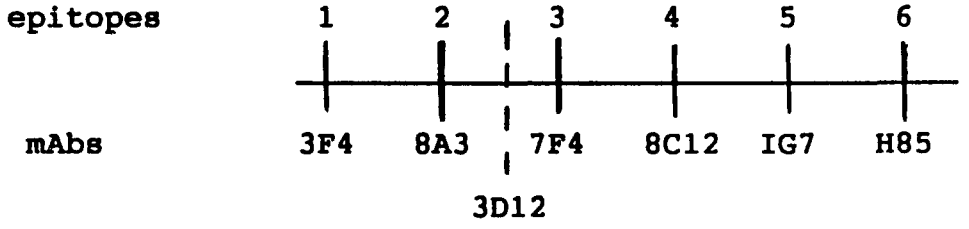
Figure 3. Displacement in the stacking order of mAbs 8A3 and 7F4 by mAb 3D12. The binding site appears to be between epitopes 2 and 3.



4. DISCUSSION

Results show partial circumvention of binding of mAbs 8A3 and 7F4 to lectin by mAb 3D12. This suggests a unique binding site for mAb 3D12 at position 2.5, that is, between epitopes 2 and 3. This data correlates well with data obtained by Dr. Petri on partial obviation of lectin-mediated lysis protection in the presence of mAb 3D12. If mAb 3D12 has partial homology to mAbs 8A3 and 7F4, then perhaps portions of epitopes 2 and 3, bind the C5b-9 complex, serving to protect pathogenic strains from lysis. Another possibility is a separate binding site for C5b-9 between epitopes 2 and 3, and, still another possibility, the ability of mAb 3D12 to change the binding configuration for C5b-9, which changes its activity and renders the amoebae more susceptible to lysis.

The naturally occurring sequence of epitopes on the adherence lectin is shown below.



The projected attachment area of mAb 3D12, i.e., the area of the lectin responsible for circumvention of elimination by the immune system, is between epitopes 2 and 3.

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